AD_____

Award Number: DAMD17-02-1-0135

TITLE: High Throughput Synthesis and Screening for Agents Inhibiting Androgen Receptor Mediated Gene Transcription

PRINCIPAL INVESTIGATOR: Dale L. Boger, Ph.D.

CONTRACTING ORGANIZATION: The Scripps Research Institute
La Jolla, California 92307

REPORT DATE: February 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	February 2003	Annual (1 Feb 02 - 31 Jan 03)

4. TITLE AND SUBTITLE
High Throughput Synthesis and Screening for Agents Inhibiting Androgen Receptor Mediated Gene Transcription

5. FUNDING NUMBERS
DAMD17-02-1-0135

6. AUTHOR(S) :
Dale L. Boger, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Scripps Research Institute
La Jolla, California 92307

Email: boger@scripps.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract (*Maximum 200 words*)

Details of the year 1 progress on our grant to define a new treatment of therapeutic resistant prostate cancer is provided. This entails the high throughput synthesis of DNA binding agents related to distamycin, their screening for binding to androgen response elements using a new high throughput DNA binding screen we introduced, and the evaluation for inhibiting androgen receptor mediated gene transcription and the cell proliferation of a prostate cancer cell line.

14. SUBJECT TERMS:
prostate cancer, DNA minor groove binding agents, androgen receptor, androgen response elements

15. NUMBER OF PAGES
10

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction. The objective of our work is the high throughput synthesis of DNA minor groove binding agents based on the distamycin structure and their screening for inhibition of androgen receptor mediated gene transcription, which is unregulated in chemotherapeutic resistant prostate cancer and responsible for disease progression. It represents a novel and unique new target for the treatment of relapse prostate cancer where prognosis is presently very poor.

Body. The results to date in our studies are presented below and refer to Task 1–Task 4 of our proposals' statement of work.

Task 1: To synthesize chemical combinatorial libraries and compounds. Two objectives were defined in our proposal. The first was to prepare a small set of modifications to compounds **124** and **128**. The initial stage of this work has been completed with the preparation of the new derivatives shown in Figure 1. As anticipated, we have shown that their ability to bind DNA increases as the pK_a increases (more protonated and thus charged). However, we have not yet found derivatives that enter cells better than **124** and **128** and the new derivatives were not better at inhibiting androgen receptor mediated gene transcription. This work continues with additional new derivatives of **124** and **128**.

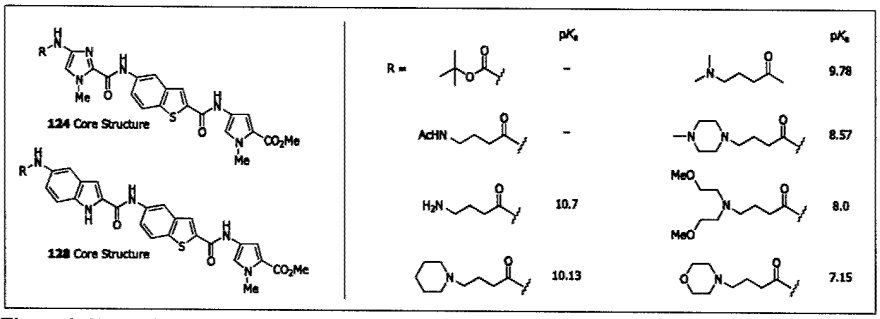


Figure 1. *N*-Terminal modifications designed to increase cell permeability.

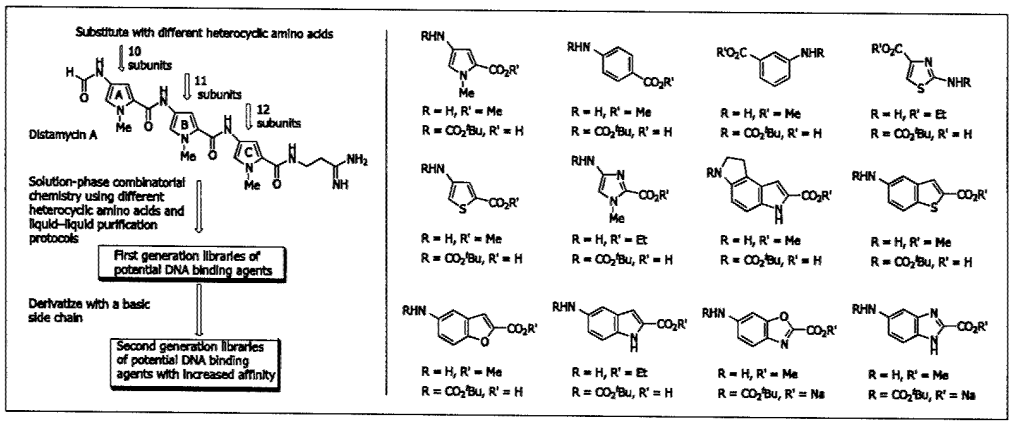


Figure 2a. Solution-phase strategy for DNA binding libraries and the amino acid subunits used in the preparation of the first library.

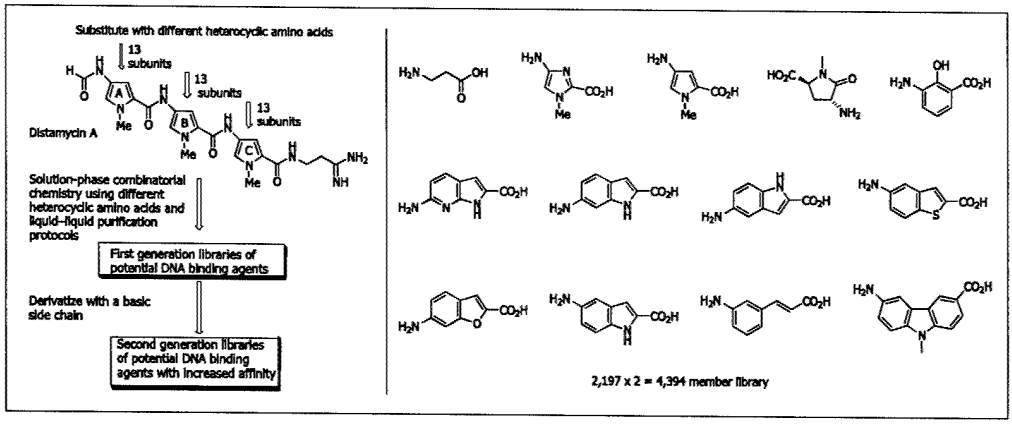


Figure 2b. Second generation DNA binding library to be generated with 13 novel subunits.

The second objective of Task 1 was to prepare additional DNA binding libraries to discover new leads. Progress on these studies has been superb. An initial library of 2,640 compounds was prepared (Figure 2a) and continues to undergo evaluation for DNA binding and inhibition of androgren receptor mediated gene transcription. A second generation library (Figure 2b) of 4,394 members is in progress and we have assembled the complete set of dimers that must now be linked to the final subunit (as a mixture of 13) to provide the completed library.

For this purpose, we also surveyed a series of building blocks that could be part of this library and

examined their ability to bind DNA in our assay entailing the displacement of ethidium bromide from hairpin DNA's. The results of this work were published and the paper detailing this work is provided in the appendix[1] (C.R. Woods, N. Faucher, B. Eschgfaller, K.W. Bair, and D. L. Boger, Synthesis and DNA binding properties of saturated distamycin analogues, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2647–2650).

Consequently, our progress on Task 1 has been outstanding.

Task 2: To identify libraries that have antitumor activity for prostate cancer cell lines and have high affinity binding to AREs.

The cytotoxic assay for the prostate cancer cell line, LNCaP, has been set up and validated.

The first library of 2,640 compounds are beginning to be tested in this cell line.

The screening of the first library of 2,640 compounds against the ARE hairpin DNAs has been performed.

Once completed, the data for the two assays will be correlated to identify active constituents that display activity against the prostate cancer cell line and bind the ARE consensus sequence.

Task 3: Define DNA binding selectivity of identified new leads.

This work has not yet begun and is waiting for the results above, although the assay is available since it was developed by our group prior to submission of this grant (Boger, et al. *J. Am. Chem. Soc.* **2001**, *123*, 5878–5891).[2]

Task 4: Determine the inhibitory effect on AR mediated gene transcription and transactivation.

As indicated in the original proposal, work on this task will be conducted at the final stages of the grant.

Key Research Accomplishments.

- Development of a novel solution-phase approach to the preparation of libraries of sequence selective DNA binding compounds (high throughput synthesis).[3]
- Development of a novel high throughput screen for establishing DNA binding selectivity or affinity. This includes the introduction of the first high throughput screen for a defined DNA sequence (i.e., androgen response element) that can control abberant gene transcription.[2]
- Definition of previously unexamined features of distamycin responsible for its DNA binding affinity (publication 1).[1]

Reportable Outcomes.

Publications

1. C. R. Woods, N. Faucher, B. Eschgfaller, K. W. Bair, and D. L. Boger, Synthesis and DNA binding properties of saturated distamycin analogues, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2647–2650.

Conclusions. The work is progressing well and requires no changes in future work. The importance of the work includes not only the potential development of a treatment for relapse (resistant) prostate cancer, but it defines a new approach to treating diseases arising from abberant gene transcription and, importantly, provides the first scientific tools to approach this problem (high throughput synthesis and screening technology for DNA binding compounds).

References.

1. C. R. Woods, N. Faucher, B. Eschgfaller, K. W. Bair, and D. L. Boger, Synthesis and DNA binding properties of saturated distamycin analogues, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2647–2650.
2. (a) D. L. Boger, B. E. Fink, S. R. Brunette, W. C. Tse, and M. P. Hedrick, A simple, high resolution method for establishing DNA binding affinity and sequence selectivity, *J. Am. Chem. Soc.* **2001**, *123*, 5878–5891. (b) D. L. Boger and W. C. Tse, Thiazole orange as the fluorescent intercalator in a high resolution FID assay for determining DNA binding affinity and sequence selectivity of small molecules, *Bioorg. Med. Chem.* **2001**, *9*, 2511–2518.
3. D. L. Boger, B. E. Fink, and M. P. Hedrick, Total synthesis of distamycin A and 2640 analogues: a solution-phase combinatorial approach to the discovery of new, bioactive DNA binding agents and development of a rapid, high-throughput screen for determining relative DNA binding affinity or DNA binding sequence selectivity, *J. Am. Chem. Soc.* **2000**, *122*, 6382–6394.

Appendix. Attached

Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2647–2650

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and DNA Binding Properties of Saturated Distamycin Analogues

Craig R. Woods,[a] Nicolas Faucher,[a] Bernd Eschgfaller,[b] Kenneth W. Bair[b,*] and Dale L. Boger[a,*]

[a]*Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA*

[b]*Novartis Institute for Biomedical Research, Novartis Pharmaceuticals Corporation—Oncology, 556 Morris Avenue, Summit, NJ 07901-1398, USA*

Received 8 April 2002; accepted 28 May 2002

Abstract—A series of saturated heterocyclic analogues of distamycin were prepared and examined. A fluorescent intercalator displacement (FID) assay conducted on p[dA]–p[dT] DNA to obtain C_{50} values and a hairpin deoxyoligonucleotide containing an A/T-rich binding site was used to evaluate DNA binding affinity. It is observed that saturated heterocycles greatly reduce the DNA binding relative to distamycin.

Polyamides composed of *N*-methylpyrrole (Py), *N*-methylimidazole (Im), and a growing set of structural analogues bind in the DNA minor groove with predictable sequence selectivity and high affinities.[1] Several factors contribute to the success of polyamide binding including hydrogen bonding, curvature, Van der Waals contacts, and charged end groups. Each component has been explored through the preparation of analogous systems designed to probe the magnitude, requirements, and relative importance of each feature. Yet, the binding effectiveness for polyamides lacking the π-system of the integral heteroaromatic rings has been relatively unexplored. Herein we report the synthesis and evaluation of a series of saturated cyclic polyamides **1–5** (Fig. 1), based on the known, effective DNA minor groove binding agent distamycin.[2] Saturation removes the π-system, alters the conformation, and increases the thickness of the compound, yet maintains the relative structure of distamycin and was used to assess the importance of the π-system. A fluorescent intercalator displacement (FID) assay conducted on p[dA]–p[dT] DNA to obtain C_{50} values and a hairpin deoxyoligonucleotide containing an A/T-rich binding site was used to evaluate DNA their binding affinity.

A solution-phase synthesis of the polyamides was conducted using a series of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) mediated coupling reactions as described previously[3] where workup, isolation, and purification could be addressed principally by liquid–liquid acid–base extractions. Each series of analogues (**1–5**) are discussed separately below.

N-CBz pyrrolidine based system (1a and b). N-Boc deprotection of **6**[4] (HCl–EtOAc) provided the amine **7** as the hydrochloride salt and methyl ester hydrolysis of **6**[4](LiOH, THF–MeOH–H_2O) provided carboxylic acid **8** (Scheme 1).

After deprotection, **7** and **8** were coupled to produce dimer **9**[5] (EDCI, HOBt, DMF, 25 °C, 13–20 h, 66%)

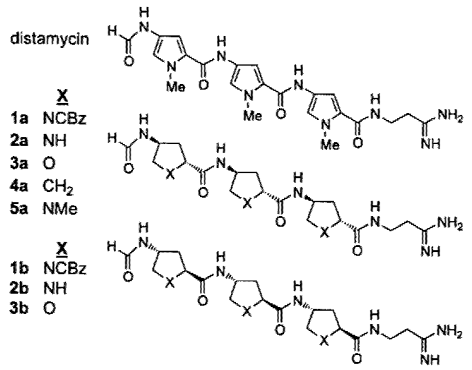
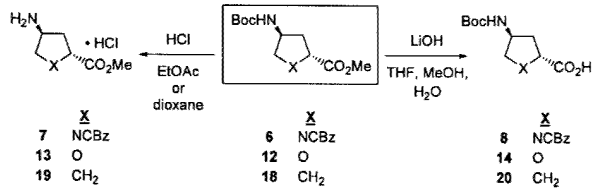


Figure 1. Distamycin and saturated heterocyclic polyamides **1–5**.

*Corresponding author. Tel.: +1-858-784-7522; fax: +1-858-784-7550; e-mail: boger@scripps.edu

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00467-5



Scheme 1.

(Scheme 2). N-Boc deprotection of **9** (HCl–EtOAc, 25 °C, 1 h) and coupling with **8** (EDCI, HOAt, DMF, 25 °C, 20 h, 73%) provided trimer **10**.[6] The methyl ester of **10** was then hydrolyzed (2.5 equiv NaOH, THF–H_2O, 1.5 h, 91%) to give the free carboxylic acid which was coupled with 3-aminopropionitrile to afford **11**[7] (EDCI, HOAt, DMF, 25 °C, 20 h, 75%). End group functionalization of **11** was accomplished by simultaneous N-Boc deprotection and imidate formation (HCl–EtOH), amidine formation (NH_3–EtOH, 25 °C, 2–24 h), and *N*-formylation (*N*-formyl imidazole, THF–MeOH, −40 °C, 2 h) without purification of the intermediates yielding **1a**[8] in 56% over the three steps. The enantiomer **1b**[8] was produced in the same manner starting from the enantiomer of **6**.[4]

Pyrrolidine based system (2a and b). Several hydrogenolysis conditions for N-CBz deprotection of **1a** and **b** were examined to produce **2a** and **b** (Table 1, entries 1–5) before efficient conditions were found (H_2, 10% Pd/C, 10 equiv TFA, MeOH, 25 °C, 4 h, entry 6). Thus, although N-CBz deprotection was slow and typically problematic, acid catalysis with inclusion of TFA provided **2** in good conversions.

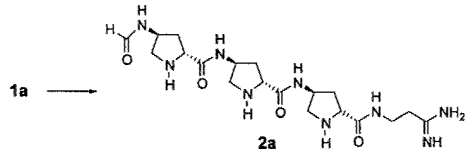
Furan based system (3a and b). Transformation of the monomer **12**[4] and its enantiomer into their respective functionalized trimers **3a**[9] and **b**[12] was performed using the strategy detailed above for **1a** and **b** (Schemes 1 and 2). However, the tetrahydrofuran-based compounds proved highly water soluble. Thus, removal of excess starting materials, reagents, and side products by simple acid–base extraction was not possible and chromatographic purifications were required.

Cyclopentane based system (4a). Transformation of the monomer **18**[4] into **4a** was performed uneventfully using the strategy detailed above (Schemes 1 and 2).

N-Me pyrrolidine based system (5a). N-Boc deprotection (HCl–dioxane) of **24**[4] provided the amine **25** as the hydrochloride salt and methyl ester hydrolysis of **24**[4] (LiOH, THF–MeOH) provided the lithium carboxylate **26**[13] (Scheme 3).

Following the strategy detailed above, the lithium salt **26** was used to provide trimer **28** (Scheme 4). However, coupling of the carboxylic acid derived from **28** with 3-aminopropionitrile afforded **29** in only moderate yield

Table 1. Hydrogenolysis conditions for **1a** using 10% Pd/C

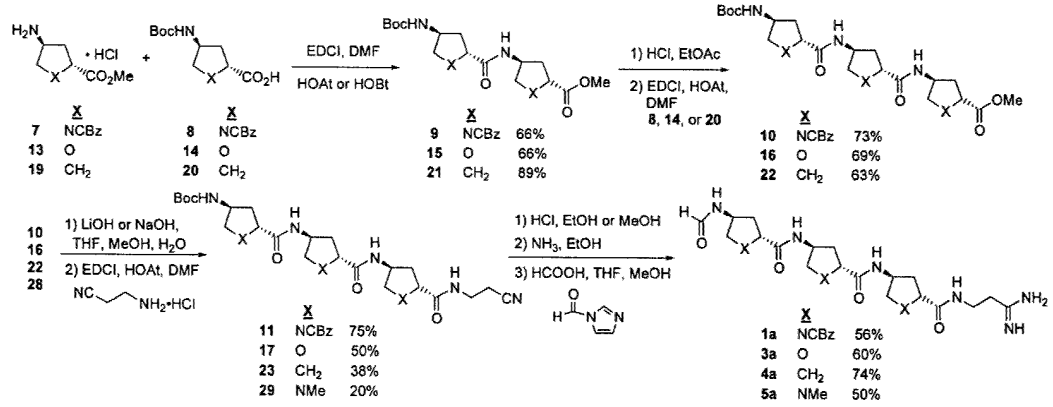


Entry	Conditions	Result
1	H_2, MeOH, 25 °C, 24–48 h	No reaction
2	H_2, MeOH, 2 N HCl, 25 °C, 24 h[10]	Decomposition
3	4% HCOOH, MeOH, 25 °C, 14 h[11]	Decomposition
4	H_2, acetone–MeOH, 25 °C, 24 h[12]	No reaction
5	H_2, 2 equiv HOAc, MeOH, 25 °C, 24 h	No reaction
6	H_2, 10 equiv TFA, MeOH, 25 °C, 4 h	**2a**, 64%

Table 2. C_{50} values

Compd	C_{50} (μM)
Distamycin	0.46
1a	4.70
1b	5.10
2a	6.37
2b	8.55
3a	5.40
3b	5.50
4a	7.91
5a	6.70

p[dA]–p[dT] 8.8 μM bp, EtBr 4.4 μM.



Scheme 2.

(20%, Scheme 2). Consequently, an alternative convergent synthesis was used to provide **29** (Scheme 4). Coupling **26** with 3-aminopropionitrile (HATU, *i*-Pr_2NEt, DMF, 25 °C, 80%) yielded nitrile monomer **30**, which was subsequently N-Boc deprotected (HCl–EtOAc) and coupled with the carboxylic acid derived from **27** (LiOH, THF–MeOH–H_2O) using standard coupling conditions (HATU, *i*-Pr_2NEt, DMF, 25 °C, 60%) to provide **29** in a more satisfactory yield. Treatment of **29** with freshly prepared 8 N HCl–EtOH led to N-Boc deprotection and imidate formation. Subsequent treatment of the imidate with NH_3–EtOH provided the corresponding amidine which was formylated using *N*-formyl imidazole freshly prepared by treating carbonyldiimidazole with formic acid to provide **5a**.[14]

Initial assessment of DNA binding. The compounds **1–5** were initially screened for DNA binding using p[dA]–p[dT] in a fluorescence intercalator displacement (FID) assay.[15] The relative binding affinities were established by monitoring the loss of fluorescence derived from titration displacement of prebound ethidium bromide from p[dA]–d[dT]. A comparison the compound concentration required for 50% displacement of the ethidium bromide (C_{50}) revealed that none of the saturated analogues compared well to distamycin (Table 2). The best of the analogues, N-CBz pyrrolidine **1a**, required a 10-fold greater concentration relative to distamycin (4.70 vs 0.46 μM), while the remaining analogues required even higher concentrations (5.10–8.55 μM). Interestingly, one stereochemical configuration, $(R)_{C\text{-}ring}(S)_{N\text{-}ring}$ (the **a** analogues), consistently performed better than their respective enantiomers.

Binding to a hairpin deoxyoligonucleotide containing an A/T-rich binding site. In an attempt to assess binding constant comparisons of **1–5**, a FID titration assay was performed using a DNA hairpin with an A/T-rich binding site of 5 bp. As above, the analogues exhibited low binding affinity toward the hairpin DNA, yielding flat fluorescence reduction curves too shallow for accurate Scatchard analysis.[16] Qualitatively, their behavior can be compared using the ratio of fluorescence present before ($F_{100\%}$) and after the addition of one equivalent of agent (F_1),[17] using the $F_1/F_{100\%}$ value (Table 3). With distamycin, a $F_1/F_{100\%}$ of 0.48 is observed, whereas much higher values are observed with the saturated systems (0.77–0.92). The trends for hairpin and p[dA]–p[dT] DNA binding ($F_1/F_{100\%}$ versus C_{50}) are similar with the N-CBz derivatives (**1a** and **b**) exhibiting the better binding properties than the remaining analogues. However, additional insights are difficult to extrapolate due to the low binding ability.

Discussion

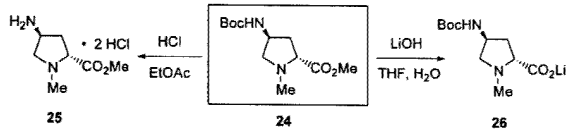
One major impact of saturating the distamycin pyrrole rings with **2** or **5** is the introduction of three additional basic nitrogens. Those of **2** are present as secondary amines while those of **5** are tertiary amines. Although not observed, one might have suspected that pH 7 protonation of these amines might provide positively charged centers that would benefit from stabilizing electrostatic interactions with DNA enhancing their affinity. In addition, the comparable behavior of **2** and **5** with either **3** or **4**, suggest that the presence of the basic amines do not enhance or inhibit binding relative to the impact of introduction of the saturated versus unsaturated

Table 3. $F_1/F_{100\%}$ values of distamycin agents **1–5** with hairpin DNA

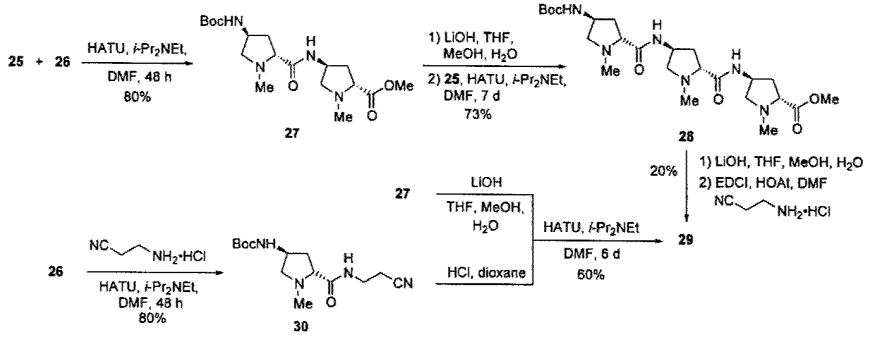
5′-C-G-A-A-A-A-A-C-A-A-A
3′-G-C-T-T-T-T-T-G-A-A

Compd	$F_1 / F_{100\%}$[a]	Curve description
Distamycin	0.48	Good
1a	0.88	Flat
1b	0.77	Flat
2a	0.90	Flat
2b	0.88	Flat
3a	0.90	Flat
3b	0.90	Flat
4a	0.92	Flat
5a	0.83	Flat

[a] $F_1/F_{100\%}$ is the ratio of fluorescence before ($F_{100\%}$) and after the addition of 1 equivalent of agent (F_1).



Scheme 3.



Scheme 4.

heterocycles. Interestingly, the most effective analogue in the series is **1** bearing the hydrophobic N-CBz protecting group. However, even **1** would not be considered an effective DNA binding agent. Finally, the all carbon cyclopentyl-based analogue **4** emerged in the DNA binding assays as the poorest analogue in the series.

Although several features imparted by the structural changes could be responsible for the poor binding of **1–5**, the magnitude of the reductions is notable. From molecular modeling studies, a minor groove complementary shape, hydrogen bonding, and stabilizing electrostatic interactions analogous to those observed with distamycin are possible with **1–5** and the hydrophobic character covered by the series brackets that of distamycin. Nonetheless, no saturated analogue in the series came close to the DNA binding affinity observed with distamycin illustrating the importance of the unsaturated heterocycles.[18]

Acknowledgements

We gratefully acknowledge the financial support of the National Institutes of Health (CA41986, CA78045), Novartis, and the Department of Army (DAMD17–02–1-0135).

References and Notes

1. (a) Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215. (b) Neidle, S. *Nat. Prod. Rep.* **2001**, *18*, 291.
2. Johnson, D. S.; Boger, D. L. In *DNA Binding Agents*; Murakami, Y., Ed.; Comprehensive Supramolecular Chemistry; Elsevier Science: New York, 1996; Vol. 4 p 81.
3. Boger, D. L.; Fink, B. E.; Hedrick, M. P. *J. Am. Chem. Soc.* **2000**, *122*, 6382.
4. Experimental details and characterization for intermediates and final products can be provided upon request.
5. For **9a**, ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.66 (m, 1H), 7.40–7.30 (m, 10H), 6.34 (brs, 1H), 5.13–4.95 (m, 4H), 4.47–4.24 (m, 4H), 3.79 and 3.39 (s, 3H), 3.75 and 3.38 (m, 4H), 2.20 (m, 4H), 1.38 (s, 9H); IR (NaCl, film) υ_{max} 3315, 2975, 1700, 1644, 1531, 1417, 1356, 1169 cm^{-1}; MALDI–HRMS (DHB) *m/z* 647.2686 ($M+Na^+$, $C_{32}H_{40}N_4O_9$ requires 647.2687); $[\alpha]_D^{25}$ +23 (*c* 1, THF). For **9b**, $[\alpha]_D^{25}$ −22 (*c* 1, THF).
6. For **10a**, ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.93 (m, 2H), 7.35–7.29 (m, 15H), 6.39 (m, 1H), 5.11–4.95 (m, 6H), 4.49–4.23 (m, 6H), 3.72 and 3.60 (s, 3H), 3.72 and 3.37 (m, 6H), 2.18 (m, 6H), 1.38 (s, 9H); IR (NaCl, film) υ_{max} 3311, 2933, 1704, 1537, 1420, 1356, 1169 cm^{-1}; MALDI–HRMS (DHB) *m/z* 893.3688 ($M+Na^+$, $C_{45}H_{54}N_6O_{12}$ requires 893.3692); $[\alpha]_D^{25}$ +24 (*c* 1, THF). For **10b**, $[\alpha]_D^{25}$ −17 (*c* 1, THF).
7. For **11a**, ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.76 (m, 1H), 7.58 (m, 2H), 7.35–7.30 (m, 15H), 6.32 (brs, 1H), 5.14–4.92 (m, 6H), 4.4–4.3 (m, 6H), 3.73 and 3.42 (m, 8H), 2.54 and 2.56 (m, 2H), 2.20 (m, 6H), 1.38 (s, 9H); IR (NaCl, film) υ_{max} 3309, 2964, 2340, 1604, 1539, 1420, 1357, 1169 cm^{-1}; MALDI–HRMS (DHB) *m/z* 931.3929 ($M+Na^+$, $C_{47}H_{56}N_8O_{11}$ requires 931.3961); $[\alpha]_D^{25}$ +29 (*c* 0.1, THF). For **11b**, $[\alpha]_D^{25}$ −29 (*c* 0.1, THF).
8. For **1a**, ^{1}H NMR (methanol-d_4, 400 MHz) δ 8.31 (brs, 2H), 8.02 (brs, 1H), 7.33 (m, 15H), 5.12–4.88 (m, 6H), 4.5–4.3 (m, 6H), 3.81 (m, 3H), 3.43 (m, 5H), 2.67 (m, 2H), 2.19 (m, 6H): IR (NaCl, film) υ_{max} 3243, 1662, 1542, 1542, 1356, 1126 cm^{-1}; MALDI–HRMS (DHB) *m/z* 854.3834 ($M+H^+$, $C_{43}H_{51}N_9O_{10}$ requires 854.3831); $[\alpha]_D^{25}$ +26 (*c* 0.1, MeOH). For **1b**, $[\alpha]_D^{25}$ −27 (*c* 0.1, MeOH).
9. For **3a**, ^{1}H NMR (methanol-d_4, 500 MHz) δ 8.02 (s, 1H), 4.47 (m, 6H), 4.11 (m, 3H), 3.76 (m, 3H), 3.47 (m, 2H), 2.69 (m, 2H), 2.31 (m, 6H); IR (NaCl, film) υ_{max} 3128, 2851, 1668, 1630, 1085 cm^{-1}; MALDI–HRMS (DHB) *m/z* 455.2244 ($M+H^+$, $C_{19}H_{30}N_6O_7$ requires 455.2249); $[\alpha]_D^{25}$ −25 (*c* 0.1, MeOH). For **3b**, $[\alpha]_D^{25}$ +22 (*c* 0.1, MeOH).
10. Oleksyszyn, J.; Boduszek, B.; Chih-Min, K.; Powers, J. C. *J. Med. Chem.* **1994**, *37*, 226.
11. ElAmin, B.; Anantharamaiah, G. M.; Royer, G. P.; Means, G. E. *J. Org. Chem.* **1979**, *44*, 3442.
12. Griffin, K. G.; Hawker, S.; Bhatti, M. A. *Chem. Ind.* **1996**, *68*, 325.
13. The lithium salt **26** was used instead of the carboxylic acid to avoid potential coupling problems with the zwitterionic form of the amino acid.
14. For **5a**, ^{1}H NMR (methanol-d_4, 400 MHz) δ 8.01 (s, 1H), 4.37 and 3.99 (m, 6H), 3.56 and 3.43 (m, 3H), 3.18 (m, 3H), 2.81 and 2.68 (m, 2H), 2.40 (m, 11H), 2.14 (m, 6H); IR (NaCl, film) υ_{max} 2939, 2852, 1643, 1659, 1580, 1256, 1107 cm^{-1}; MALDI–HRMS (DHB) *m/z* 516.3022 ($M+Na^+$, $C_{22}H_{39}N_9O_4$ requires 516.3017); $[\alpha]_D^{25}$ +88 (*c* 0.1, MeOH).
15. Boger, D. L.; Fink, B. E.; Brunette, S. R.; Tse, W. C.; Hedrick, M. P. *J. Am. Chem. Soc.* **2001**, *123*, 5878.
16. Scatchard, G. *N.Y. Acad. Sci.* **1949**, *51*, 660.
17. Addition of one equivalent of agent from the titration curves was chosen arbitrarily for the comparisons.
18. **1a**, **1b**, **3a**, and **3b** were inactive in an L1210 cytotoxic assay (IC_{50} > 10 μM).